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Suzanne Rudzinski  
Director, Transportation and Regional Programs Division  
U.S. EPA Headquarters  
6406J  
Ariel Rios Building  
1200 Pennsylvania Avenue NW  
Washington, D.C. 20460

**RE: Section 211(b) Fuels and Fuel Additives Health Effects Testing Regulation: Peer Reviewer Comments on Draft Baseline Gasoline + TAME Vapor Condensate One-Generation Reproductive Toxicity Study Report**

Dear Ms. Rudzinski:

The American Petroleum Institute (API), on behalf of the Section 211(b) Research Group (RG), is submitting independent peer reviewer comments and the RG response to those comments as required in the Alternative Tier 2 provisions of the testing regulation.

In accordance with the Alternative Tier 2 provisions, the RG has submitted the draft final report entitled *Baseline Gasoline TAME Vapor Condensate: A One-Generation Whole-Body Inhalation Reproductive Toxicity Study In Rats* to EPA for review and comment. This transmittal of independent reviewer comments and the RG response completes the information package for the EPA review of the TAME draft reproductive report. Once the RG has received EPA's comments, the draft report will be finalized with incorporation of Independent Reviewer comments as indicated, as well as EPA's comments.

When the final report of the TAME Reproductive Study is complete, it will be submitted to you at the EPA Office of Transportation and Air Quality, Transportation and Regional Programs Division, as part of the requirements of Clean Air Act Section 211(b)(2) and 211(e) (Docket No. A-90-07). If you require further information, please contact Lorraine Twerdok at 202-682-8344, or by mail at this address.

Regards,

*Lorraine E. Twerdok*

Lorraine Twerdok, Ph.D., DABT  
Administrator, 211(b) Research Group

Encl (3): Comments from Dr. Thomas Goldsworthy  
Comments from Dr. Richard Schlesinger  
RG response to comments

Cc: Joe Sopata, EPA (via email)  
Monica Alvarez, EPA (via email)  
Mike Davis, EPA (via email)  
Tom Goldsworthy (via email)

Rich Schlesinger (via email)  
211b RG Oversight and Technical Committees

## Section 211 (b) Research Group Response to Independent Peer reviewers Comments on:

### *Baseline Gasoline + TAME Vapor Condensate: A One-Generation Whole-Body Inhalation Reproductive Toxicity Study In Rats*

Provisions of the Clean Air Act, Section 211(b) Testing Program call for review of all draft final reports by two independent peer reviewers. These reviewers are Dr. Thomas Goldsworthy, Consultant, and Dr. Richard Schlesinger, Consultant, subsequently at Pace University. The present submission by the Research Group (RG) is the fourth set of comments on a series of reproductive toxicity studies on baseline gasoline and gasoline blended with oxygenates. The reproductive studies included in the 211 (b) program consist of two-generation studies on vapor condensates of baseline gasoline and gasoline containing MTBE, as well as one-generation studies on gasoline (BG) containing TAME, ETBE, DIPE, ethanol and TBA.

In the present one-generation study on baseline gasoline + TAME, rats were exposed by inhalation through a ten-week pre-mating period, during mating and gestation, and through lactation to weaning of the F1 generation offspring. Standard measures of reproductive function were made. The results indicated that it was not possible to establish a NOAEL for effect of baseline gasoline+TAME vapor condensate because decreased maternal food consumption and decreased pup weight were observed in a similar pattern over a range of doses during the lactation period. Discussion of other specific findings is contained in the report.

Overall, the reviewers concurred that the study was conducted in a "scientifically sound manner" and agreed with the report's overall conclusions. Each reviewer provided additional specific comments, which are summarized below, along with the 211 (b) Research Group's response to those comments and proposed follow up course of action, where appropriate.

#### **Dr. Schlesinger:**

Statistical Analysis: [p. 29. 2.15.1]

- Inquired why one-way ANOVA which requires multiple tests for all time points, was used rather than 2-way ANOVA which would allow evaluation of exposure groups at all times for each endpoint.  
RG response: The computerized statistical testing package employed at the laboratory is designed to perform one-way ANOVA at each time interval and is used for a range of multiple dose studies.
- Inquired if Dunnett's test was the only post-hoc test used for continuous data, and if so, why does the text refer to "additional tests"?  
RG response: Only Dunnett's test was used to compare group means to control means of continuous data. The report text will be clarified.
- Explain the criteria for using both parametric and non-parametric multiple comparison tests.  
RG response: A parametric multiple comparison test was used when data sets were homogeneous and non-parametric statistical tests were employed when data sets appear non-homogeneous.
- Why were tests conducted at both 1% and 5% levels of significance?  
RG response: These levels of significance were pre-selected since they were inherent in the laboratory's computer statistical package. Comparison at two levels of significance has been common practice in this laboratory.
- Address why "Dose groups were eliminated from statistical analysis if their standard deviation was 0 and/or  $N \leq 2$ ".  
RG response: This is a standard statement for the statistical methods section. In this study, Table 1 Summary of Survival and Pregnancy provides examples of where the n is 0 or 1 and this statement is relevant. These events are also observed in macro/micropathology incidence data. No statistical analyses are applied.
- Sperm and Motility Analysis [p. 30. 2.16.2]  
Inquired what overall statistical method was used for these data and why a pair-wise comparison was used for post-hoc testing rather than a multiple comparison test?  
RG response: The initial statistical test used for sperm evaluation was the Kruskal-Wallis non-parametric ANOVA to assess significance. This sentence was omitted from the text and will be added. If a significant difference ( $p < 0.05$ ) was observed the Mann-Whitney U-test was employed for pair-wise comparisons. Pair-wise

comparison methods are standard in the statistical package employed by this laboratory and are used for a variety of studies.

- Protocol deviations: [p. 32. 2.19]

Concern was expressed about the number of "technician-related errors" as noted in Section 2.19 (Protocol Deviations) of the report. It was suggested that this needed to be addressed with the study sponsor. Despite this concern about a "degree of technician sloppiness", Dr. Schlesinger also concluded, "there were no significant deviations from these protocols that would have affected the outcome of the study."

RG Response: Nearly all of the protocol deviations were related to isolated errors in data collection. None significantly impacted the overall results or conclusions of the study. While the absolute number of protocol deviations may seem excessive, a One-Generation Reproduction study is a considerable undertaking and involves a large number of animals. The fact that these protocol deviations were noted and documented reflects positively on the Quality Assurance practices that have been put in place by API, and at the laboratory. Nonetheless, the RG brought this issue to the attention of the laboratory to minimize future protocol deviations.

## Results

### Chamber Monitoring : [p. 33-34. 3.1]

- Note was made that the report referred to a slight inaccuracy in the calibration of the IR monitor (pg 33), and a view was expressed by Dr. Schlesinger that this would be an unacceptable source of experimental error. In addition, comment was made about the presence of particles in all of the chambers. Questions were raised about the source of these particles and why filters were not fitted to the chambers to remove these particles.

RG Response: To be precise, the report stated that the ratio of the measured to nominal concentration was not 1:1, and speculated that the cause **MAY** have been due calibration error. In any event, the inaccuracies are believed to be slight. The RG feels confident that the reported chamber concentrations accurately reflect the atmospheres to which the animals were exposed.

With regard to the presence of particles, the RG notes that the mean particle concentrations were comparable between the control and treatment group chambers. The primary purpose of making these measurements in a study of this nature (high vapor concentrations) is to make certain that vapor condensation is not occurring and to preclude the possibility of aerosol formation. The results provided in the report confirm that gasoline = TAME exposure was to vapors and not aerosols. Some particles are invariably present in chamber atmospheres, representing background levels. In fact, careful analysis of the data in the table on page 34 indicates that the particle concentrations in all of the chambers including controls ranged from about 3 to 5 micrograms per cubic meter of air. For comparison, it has been reported in EPA's PM 10 Criteria document that annual background PM 10 levels in eastern cities range from 5 to 11 micrograms / cubic meter of air. Thus, the RG believes that the particles in the chambers reflect ambient background values.

- Considered the information on p. 1 of Appendix A: In Chamber Monitoring summary unclear and inquired about the numbers on the top of the table.

RG response: Dr. Schlesinger may have received an inaccurate or illegible copy of Appendix A Table 1. The copy reviewed here appeared clear and understandable. The headings for this table are 00-4202, and 211-TAME-1G, the study number and designation 211-TAME-one generation study. The numbers across the top of the data columns are the days of exposure when physical observations were made. All animals were reported to exhibit behavior "within normal limits" while in the inhalation chambers.

- Requested that rather than state there was a "reasonably" close comparison, a more precise quantitative description of the relationship between the analyzed neat test substance and the vaporized test atmosphere be provided.

RG response: Data are in Appendix B. RG will discuss with the study director what additional information could better describe the comparability of components in the analyzed neat test material and the vaporized samples. For example, the area % of major components was consistent over 123 exposures when test atmosphere was compared with neat test material: TAME 13-14%; Isopentane 33-34%; Benzene 2-3%; n-Butane 8.5-10% and N-pentane 6.5-7.8%. The RG will suggest that "acceptably" close would be more descriptive and that an acceptable range be defined.

## Results

- Physical Observation Data [p. 35. 3.2.2; Table 2]

Noted that the number of animals reported “Normal: Within Normal Limits” showed a reduction in number at the highest exposure concentration while the report states that test animals were non-remarkable in-chamber during the exposure periods.

RG response: Animals were observed both in the inhalation chambers and in home cages. Parental animals were examined at least weekly after exposure in home cages and twice daily for mortality or severe toxic effects. Review of Table 2 indicated that the reduced number of male animals listed as “normal” as the study progressed was due to the occurrence of alopecia of the extremities and snout which was first observed in 1-2 rats in all groups including controls by the second week of exposure and increased to 5-7 rats in treated groups by the fourth week of exposure, and remained at a stable incidence to the end of the study; most affected animals were seen in the 20000mg/m<sup>3</sup> group. Females were affected to a much lesser extent with the highest incidence in controls during the pre-mating/mating period. Aside from hair loss in males, no significant physical or behavioral changes were reported. The RG will discuss adding a sentence describing the alopecia to this section with the study director.

- Feed Consumption [p.36. 3.2.5; Table 11- does not address pregnant or lactating females.]

Suggested that most effects on feed consumption occurred in the mid and high exposure levels so that “there does appear to be some consistent exposure concentration related effects.”

RG response: Dr. Schlesinger is correct that statistically significant changes in feed consumption occurred primarily in the mid and high dose groups but, as stated in the report text, there were no treatment-related patterns. Among males, statistically significant decreased feed consumption was reported on day 7-28 in high and mid dose animals and also on day 35 for low and mid dose but not high dose males; on day 61 high dose males showed increased food consumption compared to controls with decreased consumption seen again on day 91. Other days were comparable to controls. Effects in females showed statistically significantly higher feed consumption than controls at the beginning of the study in high dose females, and decreased feed consumption in mid dose females only on days 7-42 and days 63-69. Feed consumption did not decrease with increasing exposure levels in any consistent trend. The RG accepts the investigator’s assessment. Dr. Goldsworthy had no comment on this section. However, we will discuss with the study director whether a sentence should be added indicating that feed consumption changes occurred primarily in the mid dose group and among high dose males.

- Maternal Feed Consumption [p. 36. 3.2.6; Table 13]

Pointed out that, from Table 13, the high dose females showed a “consistent pattern of decreased feed consumption during lactation”.

RG response: Dr. Schlesinger is correct that high dose dams showed a statistically significant decrease in feed consumption during lactation periods, 7-14, 4-21, and 21-28. These events were discussed by the investigator in the Results section as (apparently) not relevant to exposure, because a consistent exposure-response pattern was not evident. These events correlated with a similar pattern of decreased pup weights, which also appeared to be dismissed in the Results section (p. 39). However, decreased pup weights and decreased feed consumption in lactating females was reported in the Conclusions and Summary and emphasized as contributing to the inability to establish a NOAEL for this study. The study director will be requested to review these data and rewrite the Results section to be consistent with the Conclusion and Summary, specifically citing the similar pattern of response between maternal decreased feed consumption during lactation and decreased pup weight.

- Organ Weights [p.38. 3.4.2, Table 21]

Questioned whether it was accurate for text to state that effects on liver weights in males was significant related to exposure only at the highest dose, since Table 21 showed effects at the mid dose also.

RG response: Table 21 presents absolute organ weights, organ weights relative to body wt and relative to brain wt. Absolute liver weights and liver wt relative to brain wt were statistically significantly elevated compared to controls only at the high dose, but when considered relative to body wt, high dose values were very significant ( $p < 0.01$ ) and some effect was seen in mid dose ( $p < 0.05$ ). When statistically significant effects at a dose level are only seen as relative to body or brain wt, the result at that dose level may not be recognized as toxicologically significant. Indeed, increased liver wts (absolute and relative) in the absence of corresponding histopathology findings are often considered to result from a generalized adaptive metabolic response to exposure in studies of a variety of chemicals, rather than a unique toxic effect of an individual test material. However, the study director will be requested to include the information on relative liver wt effects at the mid dose level in the text on p. 38.

Dr. Schlesinger had the impression from the text that “effects on male rat kidneys at all exposure levels were consistent with microscopic findings but these findings were discussed in terms of the highest exposure level only. What were microscopic effects at other dose levels?”

RG response: Histological examination of kidney tissue was performed only on high dose animals. Induced hyaline droplet formation indicative of light hydrocarbon nephropathy were observed only in male rats although high dose females demonstrated increased kidney wts as well. The text states only that changes in male kidney wt were consistent with microscopic findings. However, from studies with other light hydrocarbons in this program and elsewhere, it can safely be extrapolated that similar effects would be present at lower doses that induce increased male kidney wts. RG will request the study director to revise the text to make it clear that only organs of high dose animals were examined microscopically in this study.

Recommended that tables in the Postmortem section be arranged so that Tables 21 and 20 are in chronological order.

RG response: This clerical error has been corrected.

- Conclusion [p. 41. 4.0]

Noted that effects in liver and kidney wt were addressed only at the high dose level, while effects found at lower doses should also be addressed. Dr. Goldsworthy also requested that increases in male kidney wt at all doses and other organ wt changes be added to the Conclusion and Summary (p. 7).

RG response: The study director will be requested to include additional organ wt information on male kidney and liver wts in the appropriate sections.

### **Dr. Goldsworthy**

Overall, Dr. Goldsworthy had few recommendations on this study. He agreed with the conclusions and the inability to establish a NOAEL.

He recognized that increases in kidney wt in males occurred at all doses and were consistent with the microscopic findings reported in high dose males and did not request information at lower dose levels.

As indicated above, his comments agreed with Dr. Schlesinger’s comment that more detail on kidney wt and other identified weight changes should be included in the Conclusions (p.41) and Summary (p.7).